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FINAL REPORT

ROLE OF PHORETIC MITES IN THE TRANSMISSION OF THE BLUESTAIN FUNGUS, CERATOCYSTIS MINOR

by

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Ceratocystis minor (Hedgcock 1906) is the bluestain fungus associated with the southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB), and is introduced into trees by attacking beetles. *Ceratocystic minor* can be isolated from bodies of attacking *D. frontalis* (Nelson and Beal 1929, Rumbold 1931) but is not transported in the mycangium as are some other symbiotic fungi (Barras and Perry 1972). Colonization of the tree by *C. minor* is thought to aid development of beetle brood by reducing moisture content of the tree (Nelson 1934). Clark and Richmond (1977) suggest that *C. minor* also improves the beetle's diet by synthesizing certain fatty acids. But feeding in phloem bluestained by *C. minor* inhibits *D. frontalis* larval development (Barras 1970, Franklin 1970). It does not readily colonize or sporulate in beetle galleries and is not found abundantly until after brood development is completed (Oberle 1966, Barras 1970, Howe et al. 1971). Perhaps *C. minor* is masked or inhibited in active beetle galleries, possibly by the presence of other beetle-associated microorganisms or fungistatic chemicals.

Although we know *C. minor* is transmitted by SPB, we do not know how the beetle becomes inoculated with fungal spores. One possible source of inoculum is phoretic mites, especially *Tarsonemus krantzi* Smiley and Moser 1974 and *T. ips* Lindquist 1969 (Moser and Roton 1971, Moser et al. 1974). Leach et al. (1934) observed *Ceratocystis ips* on mites associated with *Ips* beetles and suggested that mites may distribute the fungus within beetle galleries. Hetrick (1949) theorized that *C. minor* was transmitted from tree to tree by mites on SPB. Tarsonemid mites feed on *C. minor* and move from areas of fungus-colonized bark to cracks in the outer bark where they attach to mature adult beetles, usually after the beetles have left their pupal cells (Roton 1978). Mites are apt to be carrying *C. minor* spores when they attach to beetles. With these facts and theories in mind, we tested the hypothesis that phoretic tarsonemid mites are a source of *C. minor* inoculum for SPB.

MATERIALS AND METHODS

We collected newly emerged SPB that flew from infested bolts to a window in the laboratory. We examined the beetles under the microscope and divided them into 2 groups: beetles without tarsonemid mites and those with 5 or more mites. After crushing each beetle in 0.25 ml of 0.85% NaCl, we plated the macerate on 2.5% malt extract (ME) agar.

Before mites became attached we collected teneral SPB adults from their mite-free pupal chambers in trees with thick (> 4 mm) and thin (< 2 mm) inner bark (Roton 1978). We crushed them in sterile saline and plated the macerate on ME agar plates. After removing adults, we sampled their mite-free pupal chambers by aseptically cutting small pieces of bark from 5 locations in the chamber and plating the bark on ME agar plates.

We collected *T. krantzi* and *T. ips* from emerging beetles and plated them on 2.5% ME agar plates. We also collected mites from bark colonized with *C. minor* by allowing them to crawl onto a sterile dissecting needle and then transferring them from the needle to ME agar plates with sterile forceps. ME agar plates were incubated at room temperature for 5-7 days and then checked for *C. minor*.

We analyzed the data using a chi-square test of independence with 100 observations in each group. Chi-square values were adjusted for continuity.

RESULTS AND DISCUSSION

Emerging SPB with phoretic *Tarsonemus* mites had a significantly greater incidence (75%) of *C. minor* than did mite-free beetles (39%, Table 1). Evidently mites are hypervectors of *C. minor*. *Tarsonemus* mites feed on black patches of *C. minor* perithecia and ascospores between the phloem and xylem (Moser et al. 1974) (Fig. 1). We isolated the fungus from almost all the mites removed from these areas of fungus-colonized bark (Table 1, Fig. 2). As mites move from areas of high ascospore concentration to the outer bark to attach to the beetle (Roton 1978), they carry *C. minor* spores with them. Of the mites removed from emerging beetles, 59% carried *C. minor* (Table 1). So *C. minor* ascospores remain on the mite after it has attached to the beetle.

Ceratocystis minor is most evident as black patches of perithecia at the interface between the phloem and xylem (Fig. 1). Because beetle larvae normally move to the outer bark to pupate, they do not touch these areas and would be less likely to be carrying *C. minor* ascospores if it were not for tarsonemid mites. But, in trees with thin inner bark, beetles are forced to pupate next to the xylem where they would have a better chance of touching *C. minor* spores. To find out whether this assumption is true, we compared the incidence of *C. minor* on mite-free beetles from trees with thin bark to the incidence on those from trees with thick bark. We found that teneral adults removed from thin bark trees had significantly more *C. minor* (49%) than did beetles from trees with thick bark (18%)--(Table 1).

Although teneral adults removed from thin-bark trees were more apt to carry *C. minor* than were beetles from thick-bark trees, incidence of *C. minor* in their pupal chambers did not differ significantly (Table 1). So *C. minor* must colonize equally the bark from thin- and thick-bark trees. We did not find perithecia in pupal chambers of thick-bark trees. Therefore, the fungus cultured from these pupal chambers was probably mycelial or conidial. Because the sticky ascospore is the propagule most likely to be picked up by the beetle, the absence of ascospore-producing perithecia explains the lower incidence of *C. minor* on beetles from thick-bark trees. In thin-bark trees, perithecia were found in pupal cells because the cells are formed next to the xylem where ascospores were produced in abundance. In thin-bark trees, beetles had more *C. minor* because they were more apt to touch ascospores that would stick to them.

Though *C. minor* can be isolated from mite-free pupal chambers (Table 1), which probably are also a source of fungal inoculum, mites increase the chance that beetles will carry fungal spores. This association is an interesting example of a three-way mutualistism among insects, mites, and fungi. The insect carries both the mite and the fungus. The fungus is food for the mite and probably aids beetle development by killing the tree rapidly. And the mite assures transmission of the fungus.

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Table 1.--Incidence of *C. minor* with *D. frontalis* and *Tarsonemus* mites

Type of Material Cultured	No. Cultured	% Positive for *C. minor*	Significant X^2 ($P < 0.05$)
Emerging beetles:			
with mites	100	75	
without mites	100	39	Yes
Teneral adults from:			
thick bark	100	18	
thin bark	100	49	Yes
Pupal chambers from:			
thick bark	100	59	
thin bark	100	54	No
Tarsonemus mites:			
phoretic	100	59	
from fungus-colonized bark	100	93	Yes

FIGURE LEGENDS

Fig. 1. A bolt of loblolly pine naturally infested with SPB. Note black patches of *C. minor*.

Fig. 2. Scanning electron micrograph showing ascospores of *C. minor* on a unidentified immature mite found on a patch of *C. minor*.